

# The Effect of Acylation on the Inhibition of HT-29 Cancer Cell Proliferation by Anthocyanin Pigments

Jennifer A. Willig\*, Joshua A. Bomser\*\*, M. Monica Giusti\*

## ABSTRACT

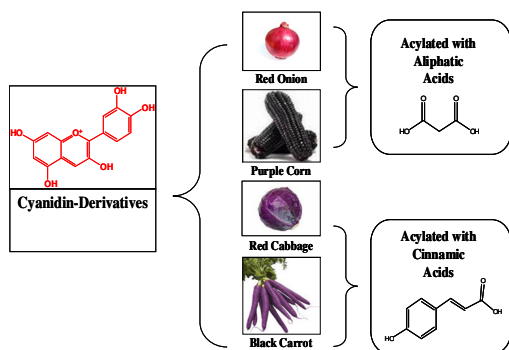
Anthocyanins are the red, purple, and blue pigments found in many fruits and vegetables. Research shows that anthocyanin-rich extracts can slow the growth of colon cancer cells. Acylated anthocyanins exhibit increased stability in food matrices as compared to non-acylated anthocyanins, and can be used as food colorants. With the addition of acylation comes added protection from environmental conditions, but acylation may affect anthocyanins bioavailability and bioactivity.

The objective of this study was to evaluate the role of acylation on anthocyanin chemoprotective properties. Acylated cyanidin derivatives were extracted from red onion, purple corn (anthocyanins acylated with aliphatic acids), red cabbage and black carrot (anthocyanins acylated with cinnamic acids). Non-acylated anthocyanins were obtained by saponification of these materials. Chemoprotective properties of the extracts were tested on human colon adenocarcinoma cells (HT-29). Growth inhibition was measured using the sulforhodamine B assay.

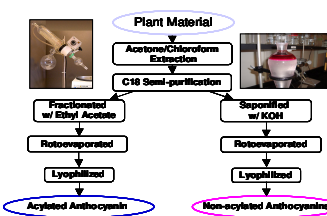
Saponification increased or maintained anthocyanin chemoprotective abilities. Results suggest that acylation and glycosylation patterns impact HT-29 cell inhibition. For example, at a dose of 100µg/ml, acylated and non-acylated red onion anthocyanins showed 120.3 and 92.5 percent inhibition respectively; where acylated and non-acylated red cabbage anthocyanins showed 20.4 and 24.7 percent growth inhibition respectively.

Both the acylated and the non-acylated anthocyanin extracts displayed inhibition properties when added to human adenocarcinoma cells (HT-29). However, due to increased stability, acylated anthocyanin extracts may impart a higher level of chemoprevention than non-acylated anthocyanin extracts and also may be more widely used by the food industry in the production of functional foods.

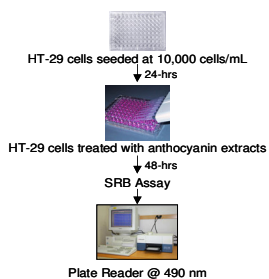
## MATERIALS AND METHODS



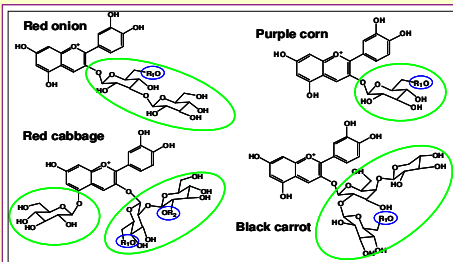
### Anthocyanin Extract Preparation



### HT-29 Cancer Cell Treatment

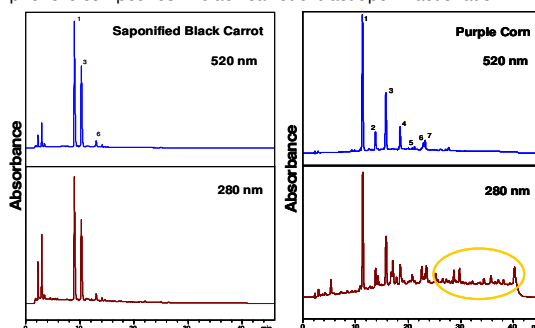


## RESULTS AND DISCUSSION

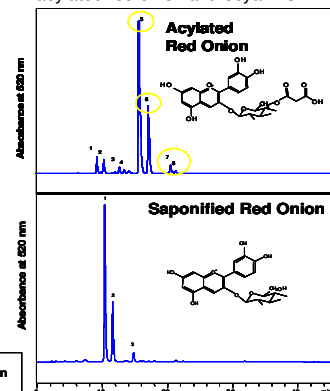


**Figure 1:** Varying acylation and glycosylation patterns for red onion, purple corn, red cabbage and black carrot anthocyanin extracts used for the treatment of HT-29 colon cancer cells.

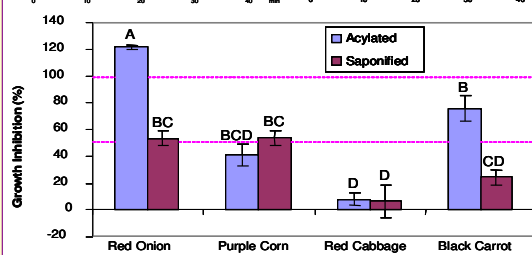
**Figure 3:** HPLC chromatograms at 520nm and 280nm displaying the successful removal of non-anthocyanin phenolic compounds in black carrot extract upon fractionation.



**Figure 2:** HPLC chromatogram at 520nm demonstrating the successful saponification of acylated red onion anthocyanins.



**Figure 4:** HPLC chromatograms at 520nm and 280 nm showing the presence of non-anthocyanin phenolic compounds found in purple corn extract upon fractionation.



**Figure 5:** Inhibition of HT-29 colon cancer cell proliferation when treated at 50µg/mL cyanidin-3-glucoside equivalents with red onion, purple corn, red cabbage, and black carrot anthocyanin extracts. Red onion showed cytotoxic effects when acylated, purple corn exhibited higher growth inhibition with saponified samples, red cabbage showed low growth inhibition, and black carrot displayed higher growth inhibition with the acylated anthocyanins.

## CONCLUSIONS

The results of this project provided evidence that anthocyanin chemical structure plays an important role in HT-29 colon cancer cell growth inhibition. Anthocyanin acylation either increased or maintained the inhibition of HT-29 cell proliferation. Anthocyanins acylated with aliphatic and cinnamic acids exhibited a different chemoprotective effect depending on the aglycone and glycosylation patterns. Also, non-anthocyanin phenolic groups may impact the chemoprotective abilities of anthocyanins. Acylated anthocyanins used as colorants may impart chemoprotective properties; however, further research is needed to better understand the impact of acylation and glycosylation on anthocyanin chemoprevention.

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